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A PRELIMINARY ACCOUNT OF SOME CYTOLOGICAL CHANGES ACCOMPANYING DESICCATION.

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The ability of certain rotifers, tardigrades and nematode worms to withstand periods of desiccation has been a subject of investigation for many biologists throughout a period of more than two hundred years. Beginning with von Leeuwenhoek in 1701 and extending to the present time, researches have been carried on at intervals, in the case of the Bdelloid rotifers, with the object of determining whether or not these animals can undergo a true desiccation. The results of the several authors have shown a striking variance, in fact in some cases the conclusions of one worker have been directly opposed to those of some other who used the same species of animal in his experiments. The latest publication upon this subject is that of Jacobs ('09) who worked on the Bdelloid rotifer, *Philodina roseola*. He concludes, after prolonged experimentation and as a result of chemical and physical tests as well as by other indirect methods, that this animal undergoes a true desiccation; that at all times the cuticle is freely permeable to water and gases and that no evidence of a waterproof cyst can be found. He notes further that desiccation is usually followed by a period of reproductive activity. The foregoing conclusions together with others not bearing directly upon the subject of this paper have been confirmed during the course of this study.

Jacobs, while conducting his investigation in a very thorough manner, made no attempt to determine, from a histological or cytological point of view, the condition of the tissues of the desiccated animals as compared with those in the normal individual. To the knowledge of the author no comparison of this sort has been attempted by any investigator up to the present time. At the suggestion of Dr. E. G. Conklin, I have undertaken an inquiry into this last question and present here a brief account

of the results obtained to date. A more detailed account based upon further work will appear in a future publication.

The anatomy of the Philodinidæ has been described by Janson (1893). The most conspicuous organs in the living animal are those of the reproductive and the alimentary systems. The latter begins with the buccal cavity at the base of the trochal organs. This narrows down to a short tube leading to the mastax. The mastax, with the digestive glands surrounding it, is followed by the thin-walled esophagus which leads into the thick-walled stomach. The posterior end of the latter is continued by the "*blasendarm*" which leads to the cloaca and thence to the anus. The reproductive organs consist of two more or less spindle-shaped bodies lying on either side of the stomach. These are the ovaries and the vitellaria and they may at all times be recognized by their prominent nuclei. The nephridia with their flame cells are easily observed in the living animal. In the foot are found the glands which secrete a substance which enables the animal to adhere to different objects. The head region contains several large coronal cells with large nuclei.

A cross section of a normal extended *P. roseola* through the mid-body region is shown in Fig. 1. The vitellaria have enormous nuclei, consisting of a central karyosome surrounded by a clear homogeneous area and peripherally by a distinct nuclear membrane. This is the "nucleolar nucleus" of Carnoy and this type of nucleus is characteristic of the greater part of the cells of the animal. The cytoplasm of the vitellarium, which is syncytial in nature, is made up of granules of varying sizes and these granules appear in different patterns particularly when a variety of fixatives are used; it has quite an affinity for nuclear stains as is usual with yolk structures. The cytoplasm of the ovary does not differ essentially from that of the vitellarium. Ovary and vitellarium are each surrounded by a thin membrane. Figs. 1 and 4 show characteristic sections through normal reproductive glands.

The cytological structure of the stomach of the Philodinidæ has been described by Zelinka ('86) in the case of *Callidina symbiotica*, by Janson ('93), and more recently by de Beauchamp ('09), in the case of *Callidina socialis*. The lumen, the position

of which in the stomach tissue is not constant, is lined with a heavy ciliated cuticula. Just beneath the cuticula are found longitudinal muscle fibers arranged at regular intervals. The part of the stomach outside the thin layer of muscle fibers is syncytial in nature. With the iron-hæmatoxylin-eosin-lichtgrün stain of de Beauchamp three elements may be distinguished, (1) nuclei having, in general, the same structure as those described for the vitellarium; (2) densely staining granules of great size, not surrounded by a clear area or membrane. These are probably aggregations of food material. (3) Vacuoles of greater or less size which stain with lichtgrün. These last are probably globules of excretory material as de Beauchamp has pointed out. In the latter's description of the stomach of *Callidina socialis* he says that the stomach is not surrounded by an "individualized membrane" but only by a layer of protoplasm which projects at the periphery. In *P. roseola*, as far as I have been able to determine, a true membrane is present (Fig. 1).

The skin of *P. roseola* has practically the same structure as that of other rotifers. It consists of two layers, cuticula and hypodermis. The former is the more densely staining layer and is composed of fine granules closely packed together; the latter is a finely reticulated plasma layer in which cell boundaries cannot be distinguished and in which nuclei are found scattered at irregular intervals. The skin is pliable and may be readily folded at any point. It is difficult to obtain sections in which one or more of these folds do not appear.

The brain of *P. roseola* is of an elongated triangular shape and lies in front of and slightly above the mastax. Zelinka ('88), in the case of *Discopus synaptæ*, has figured the brain as a syncytium in which the nuclei are closely packed together about the periphery, while in the central part is found the "*punksubstanz*," a finely granular portion without nuclei. In the greater number of cases I have been able to distinguish definite cell boundaries in the case of the cells forming the peripheral layer of the brain of *P. roseola*. The nuclei of these cells are uniformly circular in section and contain a small amount of chromatin scattered in irregular masses through a homogeneous nuclear plasm. The cytoplasm is homogeneous and has the appearance of a colorless

fluid. The "*punktsubstanz*" lies approximately at the center of the organ and is granular in structure.

In the fully extended living *Philodina* the ciliated or trochal discs are prominent at the anterior end of the body. The cilia upon these discs by their successive beating give the effect of a revolving wheel. When the animal is disturbed the discs are folded and retracted into the pharyngeal region where they may be observed as oval patches. The alimentary canal is also ciliated throughout almost its entire length.

Of the glandular structures, other than those employed for reproduction, the slime glands of the foot are perhaps most easily seen. These consist of rows of cells whose cytoplasm is alveolar or finely reticular. The nuclei are large and may sometimes be seen in the living animal. The digestive glands in the region of the mastax are similar to the foot glands in structure and staining qualities.

The changes in cell organization which accompany the process of desiccation are fairly uniform in result for all the tissues. Although slight variations have been observed, these are differences of degree and not of kind. Since the cellular elements are larger and hence more easy to observe in the vitellarium, this organ will be considered first.

In a section of the vitellarium of a desiccated *P. roseola* the most noticeable difference from the conditions which are present in the normal tissues are seen in the nucleus. Normally, as was stated before, the nuclear membrane, though definite, is not very thick. Just within the membrane is a ring of homogeneous ground substance or nuclear sap. In the center of the nucleus is found the large, densely staining karyosome. In the dried animal these conditions are exactly reversed. The karyosome may disappear entirely but if this extreme condition does not come about, the structure which remains in the position of the karyosome is similar neither in shape nor in staining qualities to the original element. In extreme cases the central area of the nucleus in the dried organ has exactly the same appearance as the clear area of the normal nucleus. The nuclear membrane becomes heavy and has the appearance of a thick ring (Fig. 5). In most cases it appears to be composed of fine granules closely

packed together. Under conditions mentioned hereafter this granular appearance may give place to a dense homogeneous black ring (iron-hæmatoxylin preparations) staining exactly like the normal karyosome. The changes in the cytoplasm, though distinct, are much less marked than the nuclear changes. With the withdrawal of water the cytoplasm increases in density and loses the regular arrangement of its particles which is characteristic of the normal vitellarium (Fig. 4). The yolk granules become arranged irregularly or in small closely packed groups as in Fig. 5. The drying process causes a loss of staining power in the tissue.

The shrinkage of the cytoplasmic portion of the tissues is well demonstrated in the case of the hypodermis. Fig. 1 show as section of this layer of the skin as it appears in the animal living under normal conditions. In a section through the dried animal (Fig. 2) it will be noticed that the hypodermal layer has shrunk markedly, approaching its normal thickness only in those places where the nuclei are located. The nuclei apparently do not diminish in size and they cause a protuberance in the dried hypodermis wherever they are found. The nuclear material is redistributed in the same manner as was described for the vitellarium.

This arrangement of the nuclear elements is found in practically all the tissues of the dried animals. A detailed description of the changes in the other organs would be, for the most part, mere repetition.

As was mentioned above, the cilia in *P. roseola* are well developed, both in the head region and in the digestive tract. It would seem that a fiber of such delicate texture as that of a cilium would not long survive the effects of a removal of moisture. Such, however, is not the case. Not only do the trochal cilia escape serious injury by the desiccation process but the same is also true of those in the digestive canal. Fig. 2 shows a section cut through the infolded trochal discs of a dried animal. There is no sign of any fusion or other abnormal condition of these elements. Each cilium preserves its identity apparently as well as would those of an animal living in a natural environment.

The changes in cell structure attending recovery from desicca-

tion are almost the exact opposite of those just described. In cases where the karyosome has entirely disappeared it begins to form again in its proper position a short time after water is added to the dried animals. The thickened nuclear membrane described above shows a greater affinity for stains at this stage and gradually assumes its normal thickness. Cytoplasmic changes are quite noticeable at this time. In the vitellarium (Fig. 6) it is frequently noticed that the material surrounding the nucleus is aggregated into strands or other irregular patterns. This would seem to indicate that the cytoplasm is more freely permeable to water in certain regions than in others and that the stage represented in Fig. 7 shows a step in the gradual redistribution of extranuclear substance attending recovery from desiccation. In the case of the other organs, as before, the process of recovery is very similar. The elements are much smaller in some cases and hence more difficult to observe but the mechanism as well as the result seems to be the same.

It has been suggested to the author that the cytoplasmic and the nuclear changes taking place in dry seeds might be analogous to the ones in the rotifers just described. With this in mind, sections of the embryo of the common Indian corn, *Zea mais*, were cut, (1) at the time the seeds were fully ripened but had not become entirely dried; (2) after the seeds were thoroughly dried; and (3) after the seeds were well germinated. A section of a typical procambium cell from each of these stages is shown herewith. Fig. 14 shows a cell from a germinating embryo. It will be noticed that the cytoplasm contains many spaces filled with cell sap. The nucleus has a ring of chromatic material just within the nuclear membrane. The nucleolus is vacuolated and does not stain in the same manner as the chromatic ring at the periphery of the nucleus. The nucleolus is surrounded by a clear area which probably consists of fluid material. Fig. 15 shows the conditions which exist when the embryo is partially dried. The chromatic ring thickens, diminishing the space between it and the nucleolus. The latter becomes more compact and the vacuoles disappear. An extreme case of drying is shown in Fig. 16. The cytoplasmic granules are closely and regularly packed together. The clear space in the nucleus has disappeared

and the substance of the nucleolus has apparently diffused throughout the nuclear area.

The changes described for the drying corn cells in the last paragraph are at first sight remarkably like those occurring in the rotifer during desiccation. In both rotifer and corn the nucleus contains a nucleolus surrounded by a clear space, while around the two is a chromatic membrane of varying thickness. When water is removed the clear space around the nucleolus disappears and comes into existence again only upon the addition of water. The substance of the nucleolus in both cases diffuses toward the periphery of the nucleus leaving a more or less clear space in the center of the same. In the cytoplasm also there is a parallel between the behavior of the cells of the two forms. Loss of water is attended by shrinkage and a consequent increase in density. The cytoplasmic materials tend to gather in small lumps which remain closely packed together until moisture is again applied.

Whether the seemingly similar processes in these representatives of the plant and animal kingdom are indeed analogous can be determined only after further study.

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DESCRIPTION OF PLATE I.

FIG. 1. Cross section through the mid-body region of a normal expanded *Philodina roseola*. Leitz compensating ocular 4, obj. 2mm.

Fig. 2. Section of a rotifer kept for eighteen days previous to fixation in an evacuated calcium chloride desiccator. Leitz oc. 4, obj. 2mm.

FIG. 3. Section through a normal animal, not expanded. Leitz oc. 4, obj. 2mm.

FIG. 4. Section through the vitellarium of a normal animal. Leitz oc. 12, obj. 2mm.

FIG. 5. Section of the vitellarium of an animal dried in an evacuated desiccator for fourteen days previous to fixation. Leitz oc. 8, obj. 2mm.

FIG. 6. Longitudinal section of an animal recovering from desiccation. The rotifer was kept in an evacuated desiccator for fifteen days, then placed in water for an hour and fifteen minutes, at the end of which time it was fixed. Leitz oc. 8, obj. 2mm.

FIG. 7. Cross section of vitellarium of animal recovering from desiccation. Animal was kept in an evacuated desiccator for six days, then placed in water for one hour, at the end of which time it was fixed. Leitz oc. 8, obj. 2mm.

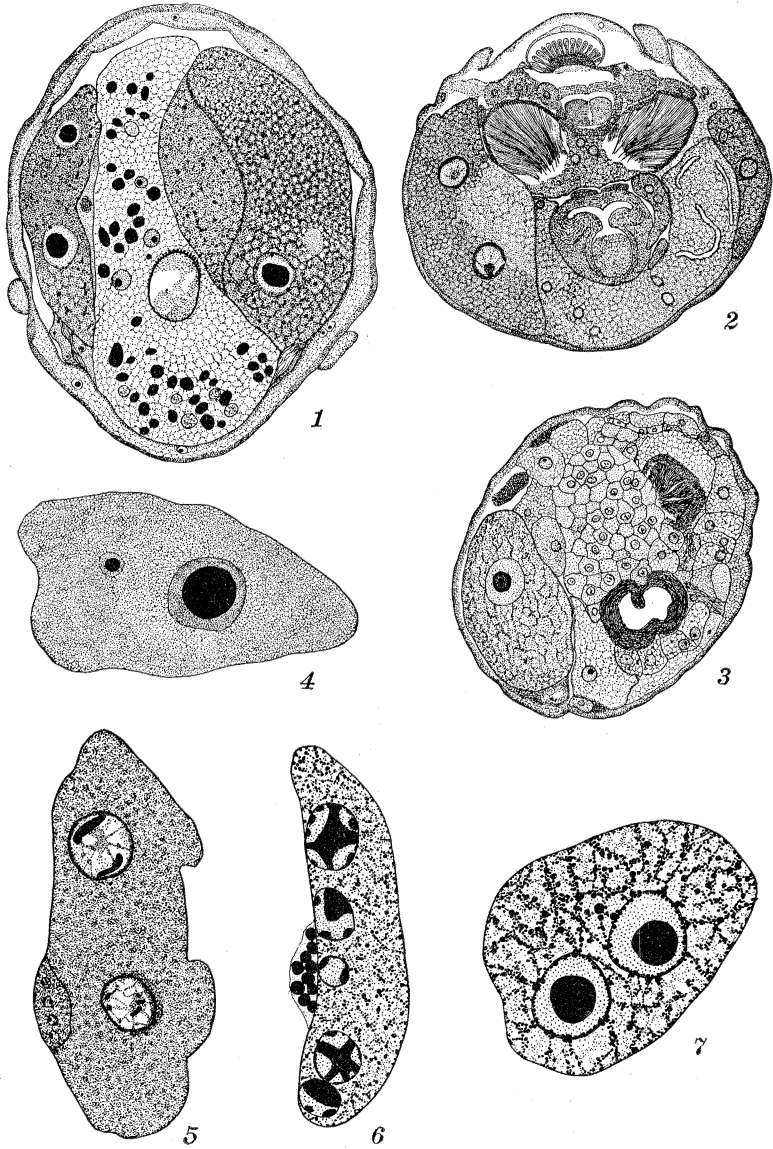


PLATE II.

FIG. 8. Section of brain of *P. roseola*. Normal active animal. Leitz oc. 8, obj. 2mm.

FIG. 9. Section of brain of a rotifer which was kept in an evacuated desiccator for fourteen days previous to the time of fixation. Leitz oc. 8, obj. 2mm.

FIG. 10. Section of brain of a rotifer which was dried twenty-four hours, put in water for one hour and then killed. Leitz oc. 8, obj. 2mm.

FIG. 11. Section of foot gland cells from a normal active animal. Leitz oc. 12, obj. 2 mm.

FIG. 12. Section of foot gland cells of a rotifer which was kept in an evacuated desiccator for fourteen days previous to the time of fixation. Leitz oc. 12, obj. 2mm.

FIG. 13. Section of foot gland cells from an animal kept fourteen days in an evacuated desiccator and then placed in water for one and one fourth hours previous to fixation. Leitz oc. 12, obj. 2 mm.

FIG. 14. Section of procambium cell from a germinating corn embryo. Leitz oc. 8, obj. 2mm.

FIG. 15. Section of procambium cell from a partially dried corn embryo. Leitz oc. 8, obj. 2mm.

FIG. 16. Section of procambium cell from a corn embryo dried for a month at room temperature. Leitz oc. 8, obj. 2 mm.

